

p21. We designed hyperbaric normoxia (HN) condition, which was 2 ATA pressurized with 10% oxygen to evaluate the effect of which means that mild hyperoxia in EP might provide the synergistic effect for cancer cell growth inhibition without a fatal damage to cells. We also studied gene expression by EP before showing growth inhibition phenotype by performing RNA-sequencing analysis of whole genome in H460 cancer cells exposed to EP for 36 hours. Among genes detected, the total of 545 genes showed 2 fold increase and 304 genes showed 2 fold decrease. And many genes were related to plasma membrane and membrane proteins including MARCH4 (membrane-associated ring finger 4) and EFNB3 (ephrin-B3) which showed most significantly changed. The other genes were also detected like genes related to ECM molecules such as MMP1 (matrix metalloproteinase 1) to cytoskeleton molecules such as EML6 (echinoderm microtubule associated protein like 6). Three genes showed time dependent changes including MMP1, P2RY6 (pyrimidinergic receptor P2Y, G-protein coupled, 6) and IL1R2 (interleukin 1 receptor, type II) by q-PCR analysis.

Keywords: elevated pressure, mild hyperoxia, Growth inhibition, non-small lung cancer cells

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[0365]

### **Influence of reductive carboxylation on redox state of cancer cells**

A. Dvorak<sup>\*1</sup>, J. Zelenka<sup>1</sup>, K. Smolkova<sup>1</sup>, L. Vitek<sup>2</sup>, P. Jezek<sup>1</sup>

<sup>1</sup>ASCR, Czech Republic, <sup>2</sup>Charles University of Prague, Czech Republic

Mitochondrial reductive carboxylation (RC) of oxoglutarate (2-OG) is indispensable metabolic pathway which plays an important role in viability of cancer cells. RC is supplemented by anaplerotic pathways - largely due to glutaminolysis. Glutamine is transformed by transaminases to 2-OG. In the RC flux, 2-OG can be converted by mitochondrial isocitrate dehydrogenase 2 (IDH2) to isocitrate or to 2-hydroxyglutarate (2-HG) with concomitant NADPH consumption. Isocitrate can be transferred to citrate by the reversed aconitase and citrate is exported from the matrix. 2-HG was claimed to be formed only by IDH2 with a R172K point mutation (1) and cannot be further metabolized. Both reactions affect reactive oxygen species (ROS) production and antioxidant protection of glutathione because of NADPH consumption. Thus we have studied redox balance and ROS production in cancer cell lines HTB-126, HepG2 and SHSY5Y and in cells where RC was inhibited by

IDH2 silencing, for all also upon hypoxia and presence of respiratory inhibitors. We have performed estimations of RC by GC-MS evaluations of <sup>13</sup>C-glutamine metabolites, namely <sup>13</sup>C citrate, malate and 2-HG. Upon hypoxia, this incorporation was much higher as compared to normoxia. RC semiquantification was taken as differences to measurements in cells with IDH2 silencing. Oligomycin increased incorporation of <sup>13</sup>C into citrate, malate and 2-HG, however, FCCP decreased it. It supports hypothesis that RC is reciprocally dependent on OXPHOS activity. We have also shown release of excess <sup>13</sup>C-glutamine metabolites to growth medium during a four days treatment with <sup>13</sup>C labeled glutamine. Also mitochondrial superoxide production and concomitant changes in NADP<sup>+</sup>/NADPH ratio were quantified in relation to IDH2 expression and activity. Thus the activity of IDH2 in matrix affects redox changes that subsequently influence redox balance, and activities of redox-sensitive mitochondrial enzymes.

1. Ward P.S., et.al., Cancer Cell 17, 225-234, March 16, 2010

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Keywords: Reductive carboxylation, Isocitrate dehydrogenase isoform 2, redox balance, GC-MS

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[0395]

### **Mitochondrial reactive oxygen species increase gastric cancer cellular invading ability**

H. Matsui\*, T. Tomita, M. Tamura  
University of Tsukuba, Japan

[Introduction] ROS play an important role for cancer cellular invasion. ROS derived from NADPH oxidase1 (NOX-1) reported to play an important role for the invasion. On the other hand, a role of mitochondrion-derived ROS for the invasion has not well investigated. Recently, we established a new cancerous cell-line RGK-1 from a rat gastric normal epithelial cell-line RGM-1. Moreover, we also established a stable clone RGK-MnSOD, which was overexpressing a manganese superoxide dismutase to scavenge mitochondrion superoxide anion. In this study, we elucidated a role of mitochondrial ROS for cancer cellular invasion.

[Methods] A rat gastric normal mucosa cell RGM-1, rat gastric cancerous cell RGK-1 which is a mutant of RGM-1 with a carcinogen MNNG, and a MnSOD overexpressing cancerous cell RGK-MnSOD were used.

The cellular ROS concentration and the cell membrane lipid peroxidation were determined using fluorescence probes HPF and DPPP, respectively. The kind of ROS was determined with EPR. The invading abilities were determined by the depth of invasion into matrigel in 48 hrs. Each cell membrane ruffling was determined to calculate the sum of the sequential 75 pictures (1 pictures/min) dark area which was the representative of membrane ruffling (Borm. EXP CELL RES 2005).

[Results and Discussion] The ROS concentration in cancerous cell was significantly higher than in normal cells, and MnSOD overexpression significantly decreased this cancer specific higher ROS concentration. The length of cancer cellular invasion into matrigel was significantly longer than that of normal, and MnSOD overexpression also significantly inhibited the invasion. The amount of ruffling in cancer cell was also larger than that of normal cells, and MnSOD overexpression decrease the cellular ruffling. These results indicated that the cancer specific high ROS concentration involved cancer cellular invading ability. Since MnSOD is a mitochondrial enzyme, the mitochondrial ROS was likely to involve the invasion ability.

Keywords: ROS, Mitochondrion, invasion, MnSOD

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[0403]

### **Trend of key markers of macrophage-derived inflammatory mediators during colorectal tumor progression**

T. Guina\*, M. Maina, A. Falcone, S. Calfapietra, F. Biasi, G. Poli

*University of Turin, Italy*

Introduction: Many studies reported a strong association between colorectal carcinoma (CRC) and inflammation. Two different macrophage phenotypes appear to be involved during the whole process of colorectal carcinogenesis, namely M1 pro-inflammatory and M2 cancer-promoting macrophages. These phenotypes, switching from M1 to M2, selectively produce different inflammatory and pro-tumoral mediators. IL(interleukin)-8 and IL-6 are commonly considered as M1 phenotype markers, while TGF(transforming growth factor) $\beta$ 1, VEGF (vascular endothelial growth factor) and MMPs (matrix metalloproteases) as M2 markers. However, the trend of serum levels of these molecule throughout the whole benign and malignant phases of colorectal carcinogenesis has never been investigated.

Methods: We enrolled twenty-eight patients with colon adenomas and sixty-eight patients with CRC at different stages who underwent surgical or endoscopic removal of the lesions. The trend of serum IL-8, IL-6, TGF $\beta$ 1, VEGF, MMPs and CRP (C-reactive protein) levels was analyzed, and TGF $\beta$ 1 receptors were evaluated in tumor tissues.

Results: Serum levels of IL-8 in CRC patients were increased from stage II, and similar behaviors of MMP-9 activity and levels were observed. The commonly used inflammatory marker CRP was compared, showing the same, but not statistically significant increase. On contrary, TGF $\beta$ 1 levels were lower at stage III, and IL-6 and VEGF levels had no significant variations. In tissue, the absence of TGF $\beta$ 1 receptors was found in 50% of adenomas, and this percentage progressively increased with malignancy. This demonstrates that loss of susceptibility to the anti-proliferative effect of TGF $\beta$ 1 is not per se sufficient to explain the shift from benign to malignant phenotype.

Discussion: M1 and M2 macrophage phenotypes were present simultaneously with no clear-cut switch from M1 to M2 phenotype. Combined quantification of certain serum markers of macrophage activation during colorectal carcinogenesis, such as IL-8 and MMP-9, together with CRP, appears to be of considerable clinical use.

Keywords: colorectal carcinoma, colonic adenomas, inflammation, tumor associated macrophages

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[0436]

### **The p38MAPK inhibition: A new combined approach to reduce neuroblastoma survival under etoposide treatment**

B. Marengo<sup>\*1,2</sup>, C. De Ciucis<sup>1</sup>, R. Ricciarelli<sup>1</sup>, A.L. Furfaro<sup>1</sup>, M. Nitti<sup>1</sup>, R. Colla<sup>1</sup>, E. Canepa<sup>1</sup>, N. Traverso<sup>1</sup>, U.M. Marinari<sup>1</sup>, M.A. Pronzato<sup>1</sup>, C. Domenicotti<sup>1</sup> et al  
<sup>1</sup>Dimes-University of Genoa, Italy, <sup>2</sup>G. Gaslini Institute, Italy

Most antineoplastic drugs induce the production of free radicals which accumulate in tumour cells, leading to oxidative stress. This damage is responsible for DNA mutations and alterations in the signaling pathways. In addition, the activation of enzymatic and non-enzymatic antioxidants contributes to the generation of a particular microenvironment that influences the behaviour of the tumour, its response to chemotherapy and the clinical outcome of the patient.